

AMENDMENTS

Amendments to the Claims:

This listing of claims will replace all prior versions and listings of claims in the application:

1-21. (Cancelled)

22. (Currently amended) A method for the synthesis and recovery of a secreted, biologically active heterologous (non-yeast) heteromultimeric polypeptide comprising at least two non-identical subunit polypeptide chains, the method comprising:

(i) producing one or more stable diploid *Pichia* cells by mating or fusing a first haploid *Pichia* cell containing a first expression construct, said first expression construct comprising nucleic acid sequences encoding for the expression of at least one subunit of said heteromultimeric polypeptide, operably linked to a first yeast promoter; and a second haploid *Pichia* cell containing a second expression construct, said second expression construct comprising nucleic acid sequences encoding for the remaining subunit(s) of said heteromultimeric polypeptide, operably linked to a second yeast promoter by mating or spheroplast fusion of haploid *Pichia* cells under conditions yielding one or more diploid *Pichia* cells, wherein said diploid cells comprise at least one expression construct encoding which eneede for at least two non-identical subunit polypeptide chains, and which stable diploid *Pichia* cells are capable of the assembly, expression and secretion of said heteropolymeric polypeptide which is comprised of said at least two non-identical subunit polypeptide chains into a culture medium when cultured in said medium under appropriate culture conditions;

(ii) culturing said diploid *Pichia* cells, or diploid progeny thereof, in a culture medium under conditions resulting in the expression, assembly and secretion of said biologically active heteromultimeric polypeptide in the culture medium; and

(iii) recovering the resultant heteromultimeric polypeptide from the culture medium, wherein the heteromultimeric polypeptide is an antibody or an antigen binding antibody fragment.

23. (Cancelled)

24. (Previously presented) The method according to claim 22, wherein said *Pichia* cells are selected from *Pichia pastoris*, *Pichia methanolica*, and *Pichia angusta*.
25. (Previously presented) The method according to claim 24, wherein said *Pichia* cells are *Pichia pastoris*.
26. (Cancelled)
27. (Previously presented) The method of claim 22, wherein said expression constructs are integrated into the genome of said diploid *Pichia* cells.
28. (Previously presented) The method of claim 22, wherein said expression constructs are contained on extrachromosomal elements.
29. (Previously presented) The method of claim 22, wherein the first or second promoters are constitutive.
30. (Previously presented) The method of claim 22, wherein the first or second promoters are inducible.
31. (Currently amended) The method of claim 22, wherein the diploid ~~yeast~~ *Pichia* cells are grown in a production media.
32. (Currently amended) The method of claim 31 [[30]], wherein said production media is a minimal media.
33. (Currently amended) The method of claim 32 [[31]], wherein said minimal media lacks selective agents.
34. (Currently amended) The method of claim 32 [[31]], wherein said minimal media lacks pre-formed amino acids or other complex biomolecules.
35. (Currently amended) The method of claim 22, wherein said diploid *Pichia* cells are grown to a ~~high cell density~~ cell density of at least about 50 g/L.
36. (Cancelled)

37. (Currently amended) The method of claim 35 [[36]], wherein said ~~high~~ cell density is at least about 100 g/L.
38. (Currently amended) The method of claim 37, wherein said ~~high~~ cell density is at least about 300 g/L.
39. (Currently amended) The method of claim 38, wherein said ~~high~~ cell density is at least about 400 g/L.
40. (Currently amended) The method of claim 39, wherein said ~~high~~ cell density is at least about 500 g/L.
41. (Currently amended) The method of claim 22, wherein said diploid *Pichia* cells are grown under conditions resulting in levels of said biologically active heteromultimeric polypeptide in the culture medium which are at least about 50 mg/L.
42. (Currently amended) The method of claim 41, wherein said diploid *Pichia* cells are grown under conditions resulting in levels of said biologically active heteromultimeric polypeptide in the culture medium which are at least about 100 mg/L.
43. (Currently amended) The method of claim 42, wherein said diploid *Pichia* cells are grown under conditions resulting in levels of said biologically active heteromultimeric polypeptide in the culture medium which are at least about 500 mg/L.
44. (Currently amended) The method of claim 43, wherein said diploid *Pichia* cells are grown under conditions resulting in levels of said biologically active heteromultimeric polypeptide in the culture medium which are at least about 1000 mg/L.

Claims 45-47. (Cancelled)

48. (Currently amended) The method of claim 22, wherein at least about 99% of said diploid *Pichia* cells comprise said expression constructs after culturing for at least 20 doublings.
49. (Currently amended) The method of claim 48, wherein at least about 99% of said diploid *Pichia* cells comprise said expression constructs after culturing for at least 50 doublings.

50. (Currently amended) The method of claim 49, wherein at least about 99% of said diploid *Pichia* cells comprise said expression constructs after culturing for at least 100 doublings.
51. (Currently amended) The method of claim 22, wherein the diploid *Pichia* cells express the heteromultimeric polypeptide at a level of expression which is reduced by not more than about 20% relative to the starting level of expression after culturing for at least 20 doublings.
52. (Currently amended) The method of claim 51, wherein the diploid *Pichia* cells express the heteromultimeric polypeptide at a level of expression which is reduced by not more than about 20% relative to the starting level of expression after culturing for at least 50 doublings.
53. (Currently amended) The method of claim 52, wherein the diploid *Pichia* cells ~~are~~ express the heteromultimeric polypeptide at a level of expression which is reduced by not more than about 20% relative to the starting level of expression after culturing for at least 100 doublings.
54. (Currently amended) The method of claim 31, wherein the diploid *Pichia* cells express the heteromultimeric polypeptide at a level of expression which is reduced by not more than about 10% relative to the starting level of expression after culturing for at least 20 doublings.
55. (Currently amended) The method of claim 31, wherein the diploid *Pichia* cells express the heteromultimeric polypeptide at a level of expression which is reduced by not more than about 5% relative to the starting level of expression after culturing for at least 20 doublings.
56. (Previously presented) The method of claim 22, wherein said culture containing said diploidal *Pichia* cells is grown at a temperature of not more than 22°C.
57. (Previously presented) A culture medium containing a stable diploid *Pichia* culture according to claim 22, wherein the culture medium comprises expression levels of said biologically active heteromultimeric polypeptide which are at least about 50 mg/liter.
58. (Previously presented) A culture medium containing a stable diploid *Pichia* culture according to claim 22, that expresses said heteromultimeric polypeptide into a culture medium, wherein the cell density of said *Pichia* diploid cells in said culture are at least about 50 g/L.